## REMARKS

Claims 27-47 were previously pending from the prior parent application (Serial No. 07/967,646, filed on October 28, 1992) of which the instant application is a file wrapper continuation (Rule 1.62). The previously pending claims, 27-47, have been canceled in favor of new claims 48-132. Accordingly, claims 48-132 are presented for further examination.

Changes to the specifications have also been effected in several instances as follows. **First,** a title of the invention has been substituted above. The new title is believed to be more descriptive of the subject matter now being pursued in claims 48-132. Furthermore, in drafting and submitting the new title, Applicants resorted to the provisions of 37 C.F.R. §1.72 and the Manual of Patent Examining Procedure (MPEP) §601 Content of Application; §606 Title of Invention; and §1302.04(a) Title of Invention. **Second,** a new section cross-referencing this application with other prior related applications has been added on page 1 in the specification. Included among the prior patent-related documents of the instant application is U.S. Patent No. 4,994,373 (issued on February 19, 1991). The new cross-reference section is believed to obviate the matter of priority under 35 U.S.C. §120. Priority under this statute had been raised in the October 2, 1995 Office Action (pages 2-3) where the Examiner astutely observed that the prior U.S. Patent Application Serial No. 07/607,347 should have been listed as having been abandoned.

**Third,** the citations for four documents cited in the seminal application in the family, U.S. Patent Application Serial No. 06/461,469 (filed on January 21, 1983) have been updated. The updated citations include seven (7) issued U.S. patents and a published PNAS scientific article. Although some may have already been submitted as exhibits in their June 2, 1994 Information Disclosure Statement Under 37 C.F.R. §§1.56 & 1.99, Applicants are nevertheless providing a copy of each of the six (6) issued U.S. patents, 4,711,955, 5,328,824, 5,449,767, 5,241,060, 5,260,433, and 4,358,535, attached hereto as Exhibits 2-7, respectively. It is believed that the foregoing updated citations will serve to improve the readability of the present disclosure by guiding future readers and Examiners to issued U.S. patent numbers, the contents of which have been incorporated into the instant

application. **Finally,** a new abstract of the disclosure (attached as Exhibit 1) is being submitted. As in the case of the new title, the new abstract (Exhibit 1) is believed to be more descriptive of the instantly claimed subject matter.

In an effort to define the instant invention more clearly, Applicants have added new claims 48-132 in place of former now canceled claims 27-47. Claims 48-99 are all drawn to a "composition of matter" which is a proper and statutorily recognized class of subject matter. See discussion below with respect to the Objection and Rejection under 35 U.S.C. §112, first paragraph, page 23, second paragraph, through page 24, penultimate paragraph. In particular, claims 48 and 77 are drawn to a composition of matter comprising a transparent non-porous or translucent non-porous system capable of retaining or containing a fluid or solution. In the case of claim 48, the system comprises a solid support to which a double-stranded oligonucleotide or polynucleotide is directly or indirectly fixed or immobilized. In the case of claim 77, the double-stranded oligonucleotide or polynucleotide is directly or indirectly fixed or immobilized to the system itself. In addition, the new composition of matter claims (48-99) as well as the rest of the claims (100-132) have been drafted to recite plural labels and signalling moieties may be incorporated into a strand of the oligonucleotide or polynucleotide (claims 48-99), one strand in the hybrid in the apparatus (claim 100), the first single-stranded oligonucleotide or polynucleotide strand in the kit (claim 101), the oligonucleotide or polynucleotide (i) in the transparent non-porous or translucent non-porous system (claims 102-131) and the oligonucleotide or polynucleotide hybrid in the apparatus (claim 132). The phrase "label or labels" and "signalling moiety or moieties" is well supported by the original disclosure, with the plural form (labels and moieties) merely capturing the disclosed feature of the present invention that multiple forms of these can be incorporated into nucleic acid.

In the case of former claim 31, the Markush members therein have been recast in accordance with the Examiner's remarks in the January 5, 1995 Office Action (see page 7, second full paragraph). The various new dependent claims which include the Markush members for the solid support or the system have been drafted also in accordance with the provisions of MPEP §803.02. Included are Markush recitations for the porous solid support (claims 51-52 and 105-106); the

non-porous solid support (claims 53-55 and 107-109); and the non-porous system (claims 78-80). In particular, Applicants have segregated the porous polymeric materials (dextran, cellulose and nitrocellulose) from the non-porous polymeric materials (polyethylene, polypropylene, polystyrene and epoxy). Support for the terms "cellulose" and "nitrocellulose" in the new dependent claims is taken from original claims 45 and 46, respectively. With regard to "polyethylene" in the new dependent claims, support is found in original claim 43. For "epoxy," support is taken from page 31, last paragraph; and original claim 66. Further embodiments for siliceous matter are found in new dependent claims 54, 79 and 108 ("siliceous material comprises glass or a glass-coated surface"). Support for "siliceous material" in the foregoing claims is found in the seminal application (page 24, lines 1 and 4-5; page 31, line 24; and original claim 4). Support for "glass-coated surface" in the foregoing claims is found at page 24, last five lines, through the first paragraph on page 25; page 27, last paragraph (see in particular, lines 18-27); and original filed claim 40.

The term "member" which had been present in former now canceled claim 32 is no longer present in its corresponding new dependent claims 56, 81 and 110. Admittedly (and as noted in the indefiniteness rejection under 35 U.S.C. §112, second paragraph), the "member" recitation in claim 32 may have been understood by the reader as referring to an additional "member" element of the invention, which otherwise is not present. Related to the aforementioned claim 32 and new claims 56, 81 and 110 are claims 57-58, 82-83 and 111-112 which recite either "wherein said well comprises a microtiter well" or "said wells in the apparatus comprise microtiter wells." The latter recitation clarifies the language in former now canceled claim 33 which read "[t]he composition according to claim 32 wherein said well comprises a microtiter well." These changes in the new claims are discussed below with respect to the rejection under 35 U.S.C. §112, second paragraph.

In the case of new claim 100 which corresponds to former now canceled claim 46, the apparatus is now defined to comprise "one or more solution containing means, each comprising a transparent non-porous or translucent non-porous device" . . . and a chemical label or labels in the second component further comprising a "signalling moiety or moieties which are capable of generating a soluble signal." These changes merely make explicit that the apparatus can take the form of separate devices, e.g., plastic or glass wells, tubes, cuvettes or arrangements of wells, tubes or cuvettes. See the instant specification, page 13, last paragraph, through page 14, first line. In effect, the language in new claim 100 adopts at least in part the Examiner's comments regarding a "plurality of devices." See the October 2, 1995 Office Action, page 4, in the discussion of the objection and rejection under 35 U.S.C. §112, first paragraph.

Further attention is drawn to new claims 63, 86 and 118 which recite that the nucleic acid "is selected from the group consisting of DNA, RNA, a DNA-RNA hybrid and a DNA-RNA chimera." Other new claims, 74-76, 97-99 and 129-131, are directed to specific techniques for detecting the soluble signal (74-75, 97-98 and 129-130) or the soluble signal itself (claims 76, 99 and 131). The latter claims define Markush members for the soluble signal ("a colored product, a chemiluminescent product and a fluorescent product") and correspond to claim 15 in U.S. Patent No. 4,994,373. The '373 patent is, of course, in the same family as the instant application and generally covers detection methods.

Finally, it should be noted that new claims 102-132 correspond in large part to former claims 28-48 whose entry was requested in Applicants' October 28, 1994 Supplemental Amendment to September 2, 1994 Amendment Under 37

C.F.R. §1.116, but denied by the Examiner.[1] See also January 5, 1995 Office Action (page 2, third paragraph, through page 4) issued in the parent application (Serial No. 07/967,646). Support for new claims 102-132 and the issue of new matter raised in the prior parent's Office Action is discussed below in connection with the objection and rejection under 35 U.S.C. §112, first paragraph. Entry of new claims 102-132 is urged in light of the remarks above, the attached claim chart (Exhibit 2) and the remarks below in response to the new matter rejection.

Applicants acknowledge with appreciation the indication in the October 2, 1995 Office Action (page 2) that previously applied rejections in the predecessor application as well as the finality of the July 25, 1994 Office Action have been withdrawn.

**The Objection and Rejection Under 35 U.S.C. §112, First Paragraph**

The specification was objected to and claims 27-47 were rejected under 35 U.S.C. § 112, first paragraph "as the disclosure, as originally filed, does not provide support for the invention as is now claimed." In the October 2, 1995 Office Action (pages 3-4), the Examiner stated:

> The preliminary amendment, filed 10/28/92, has been filed in a continuation application and therefore any added disclosure therein is NEW MATTER compared to the earlier filed application of which the instant one is also a continuation. **[1]**Claim 27 contains NEW MATTER in that a composition is therein cited without "composition" being defined or given in a written description as filed. It is noted that various probes are cited on page 9 in the first paragraph of the SUMMARY OF THE INVENTION but not compositions such as a mixture containing said probe or probes. **[2]**Additionally, the claimed composition is cited in claim 27 as comprising a system discussed above as containing NEW MATTER and wherein such a "system" also lacks written basis as filed. **[3]**Also, comparing claims 24-26 as filed to claim 27, it is clear that a

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[1]In the October 2, 1995 Office Action (page 2) the Examiner stated:

The proposed amendments, filed as after final amendments on 9/2/94 and 10/28/94, were both denied entry because both proposed amendments contain new matter as discussed in the office action, mailed 1/5/95 in the parent serial number 07/967,646. Neither of these amendments have been entered because applicants' request for a file-wrapper-continuation did not contain a request to enter either amendment and they were denied entry when their status was of after final amendments.

> solid support with a polynucleotide or oligonucleotide probe is the scope of the instant invention and not a composition "comprising" a "system" which further comprises a double-stranded oligonucleotide or polynucleotide. These three layers of subject matter go far beyond claim 24, for example, as filed, and therefore are deemed to contain NEW MATTER. **[4]**Also claims 27 and 28 contain NEW MATTER similar to non-entered claims 48 and 49 as discussed above regarding a solid support subelement in a system as submitted but not entered in the parent application. **[5]**The "same" or "different" limitations of claims 34 and 35 have not been found as filed as also the sandwich type formats of claims 36 and 37. **[6]**Similarly, the limitation directed to direct or indirect attachment of signalling moiety to the chemical label of claim 42 is NEW MATTER as being a separate limitation from that of claim 41. **[7]**The apparatus of claim 46 is not limited to a "plurality of devices" as are all of the apparatus disclosures as filed. In summary, the preliminary amendment filed 10/28/92 contains many NEW MATTER disclosures in claims 27-47 that are have no written description in the instant applicant as filed.

Because the above objection and rejection apply only to former claims 27-47, but not to claims 48-75, the entry of corresponding claims 102-132 having been requested above), Applicants would also like to address the new matter issue previously raised in the January 5, 1995 Office Action as it applies to new claims 102-132. In that Office Action (page 4), the Examiner stated:

> None of the above disclosures as filed supply written description of the system of proposed claims 48-68 regarding the specific probe plus solid support given as two component system. **[8]**Also, the open claim language term "comprises" in line 2 of claim 48 is broader than the third embodiment given in the specification on page 14 or claims 24-26, as filed, in that at the very least a polynucleotide is always fixed on the solid support as cited in claim 24 as filed. Labeled supports containing both the polynucleotide of claim 24 plus a chemically labeled probe is then the subject matter of claim 25, as filed. In summary, the scope of the above cited "comprises" is broader than the only similar embodiments where "at least" a polynucleotide is fixed onto the support with labeling only via the hybridization of a chemically labeled probe. Numerous other NEW MATTER limitations are present in the 10/28/94 amendment. **[9]**For example, a separate solid support with no limitations regarding porosity or transparency as a "subelement" in a nonporous transparent system etc. is not disclosed in the above summarized disclosure as filed as now proposed in claims 48 and 49. **[10]**The "same" or "different" materials limitations of claims 53 and 54 have not been found as filed. **[11]**The apparatus of claim 68 is not limited to a "plurality of devices" as are all of the apparatus disclosures as filed. In summary, the proposed amendment filed 10/28/94 contains NEW MATTER disclosures that are have no written description in the instant application as filed.

The objection and rejection for NEW MATTER are respectfully traversed.

In order to insure that each and every new matter issue has been properly and thoroughly addressed, Applicants have taken the liberty of inserting bold bracketed numbers before each specific issue raised in the above quoted rejections. The remarks below follow the order of the eleven (11) bold numbers inserted above.

In setting up and applying the legal standards for adequate description and new matter, several tenets have been expostulated by the courts as guides for analysis. Among these tenets are the following:

**First,** the legal test for determining compliance with the written description requirement is *whether the disclosure of an application as originally filed reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter*. See Ex parte Rohrer, 20 USPQ2d 1460 (Bd. Pat. App. & Int'f 1991); Ex parte Holt, 19 USPQ2d 1211, 1213 (Bd. Pat. App. & Int'f 1991); and Ex parte Remark, 15 USPQ2d 1498, 1506 (Bd. Pat. App. & Int'f 1990).

**Second,** it is not necessary that the claimed subject matter be described identically, but the disclosure originally filed must convey to those skilled in the art that the applicant invented the subject matter later claimed. Rohm & Haas Co. v. Mobil Oil Corp., 212 USPQ 354 (D. Del. 1981) (citing Treatise); Ex parte Rodgers, 27 USPQ2d 1738, 1743 n.11 (Bd. Pat. App. & Int'f 1992). It is not necessary that the claimed subject matter be described in *ipsis verbis* to satisfy the written description requirement of 35 U.S.C. §112. Nelson v. Bowler, 1 USPQ2d 2076, 2078 (Bd. Pat. App. & Int'f 1986).

**Third,** the inquiry into whether the description requirement is met must be determined on a case by case basis and is a question of fact . . . [T]he Examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would recognize in [applicant's] specification disclosure a description of the invention defined by the claims. Ex parte Sorensen, 3 USPQ2d 1462, 1463 (Bd. Pat. App. & Int'f 1987).

Referring now to the bold bracketed numbers above, Applicants offer the following remarks.

[1] Regarding the use of the term "composition" in claim 27 (now new claims 48 and 77) Applicants note that these claims are directed to a statutorily recognized class of subject matter, i.e., a composition of matter. As such, Applicants believe that they are entitled to claim the products at hand in the guise of composition of matter claims. Claims 48 and 77 (including claims dependent therefrom) are all directed to a composition of matter and thus conform in all respects to the statute, usage in patent practice and even constitutional history. Furthermore, patent commentators have broadly defined the term "composition of matter." In fact, Robinson defines a "composition of matter" as:

> . . . an instrument formed by the intermixture of two or more ingredients, and possessing properties which belong to none of these ingredients in their separate state. . . . The intermixture of ingredients in a composition of matter may be produced by mechanical or chemical operations, and its result may be a compound substance resolvable into its constituent elements by mechanical processes, or a new substance which can be destroyed only by chemical analysis.[2]

Walker notes that "this class [composition of matter] is a very broad one and embraces chemical compounds, mechanical or physical mixtures, alloys and a great variety of things."[3]

Applicants acknowledge the indication in the Office Action that "various probes are cited on page 9 in the first paragraph of the SUMMARY OF THE INVENTION but not compositions such as a mixture containing said probe or

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[2]W. Robinson, *The Law of Patents for Useful Inventions, pages* 278-79 (1890).

[3]A Deller, *Walker on Patents* 126-27 (2d ed. 1964). See also Diamond v. Chakrabarty, 100 S. Ct. 2204, 206 U.S.P.Q. 193 (1980), discussed § 1.02[7][d] *infra* (" 'composition of matter' has been construed consistent with its common usage to include 'all compositions of two or more substances and . . . all composite articles, whether they be the results of chemical union, or of mechanical mixture, or whether they be gases, fluids, powders, or solids.' Shell Dev. Co. v. Watson 149 F. Supp. 279, 280 (DC 1957) (citing 1 A. Deller, *Walker on Patents* §14, p. 55 (1st ed. 1937)). In choosing such expansive terms . . . modified by the comprehensive 'any,' Congress plainly contemplated that the patent laws would be given wide scope.").

probes." In response, Applicants would respectfully like to point out that that cited portion is but one portion in the disclosure at hand, and further, to do justice to the present invention, the entire disclosure should and must be viewed as a whole. In this regard, it is noted that on page 14, for example, Applicants disclose:

> A further aspect of the present invention provides **products** useful in the disclosed method for detection of a polynucleotide sequence. Among these **products** is a device containing a portion for retaining a fluid. Such portion contains an immobilized polynucleotide sequence hybridized to a polynucleotide or oligonucleotide probe. The probe, as described above, has covalently attached thereto a chemical label including a signalling moiety capable of generating a soluble signal. Also part of the device is a soluble signal, preferably a colored or fluorescent **product**, generatable by means of the signalling moiety. The portion of the device for containing the fluid is desirably a well, a tube, or a cuvette. A related **product** of the invention is an apparatus comprising a plurality of such devices for containing a fluid, in which at least one such device contains the above-described immobilized polynucleotide sequence, polynucleotide or oligonucleotide probe, signalling moiety, and soluble signal. Additionally the present invention provides for the novel **product** of a non-porous solid support to which a polynucleotide is directly fixed in hybridizable form. Such a fixed sequence may be hybridized to another polynucleotide sequence having covalently attached thereto a chemical label including a signalling moiety capable of generating a soluble signal. As indicated above, the support is preferably transparent or translucent. Such **products** could be advantageously employed in diagnostic kits and the like.

It is respectfully submitted that the above-quoted disclosure supports the composition of matter now embodied in claims 48-99. The term "product" is properly used to describe compositions of matter as well as apparatus or devices as part and parcel of the present invention.

**[2]** With respect to the term "system" in claim 27 (new claims 48, 77 and 102) Applicants respectfully point out that this term has indeed a written basis as filed. For example, in the original parent application this term is used both in the specification (page 28, first paragraph) as well as in the originally filed claims (claim 71), the latter reciting "[a] substrate in accordance with Claim 1, wherein said substrate is adapted for the transmission of light transversely through the system." Thus the use of "system" in claims 48, 77 and 102 is altogether proper and in no way is believed to encompass new matter.

[3] Concerning the issue of the subject matter in claims 24-26 vis a vis claim 27 (new claims 48 and 77) the following is noted. First of all, previously canceled claims 24-26 were directed to a solid support having directly fixed thereto a polynucleotide sequence in hybridizable form (independent claim 24 and dependent claim 26) or hybridized form (dependent claim 25). On the other hand, claims 48 and 77 are directed to a "composition which comprises a transparent non-porous or translucent non-porous system further comprising a double-stranded oligonucleotide or polynucleotide . . ." Thus the subject matter of previously canceled claims 24-26 should not be construed as limiting the subject matter of claims 27+ because the two sets of claims represent different embodiments of the present invention. To restrict the scope of the new claims by relying on the subject matter recited in an independent set of claims (24-26) would not be appropriate. Second, the issue of "composition" and "system" has been addressed in the first two points above. As indicated above, these terms and their use in the instant claims are well supported in the original disclosure and do not constitute new matter.

[4] Regarding the issue of former claims 27 and 28 (new claims 48-49) vis a vis former claims 48 and 49 (new claims 102-103) and the "solid support subelement in a system," the following is noted. New claims 48 and 49 (the latter being dependent) are directed to a composition of matter comprising a transparent or translucent non-porous system capable of retaining or containing a fluid or solution. The system comprises (i) a solid support, and (ii) a double-stranded oligonucleotide or polynucleotide which is directly or indirectly fixed or immobilized to said solid support. . . one of the strands comprising a chemical label or labels further comprising a signalling moiety which is capable of generating a soluble signal. In the case of new claims 48 and 49, both claims depend from claim 31, which ultimately depends from claim 27. Because the issue of new matter in claim 27 is believed to have been satisfactorily addressed in the first three points above, the entry of new claims 48 and 49 is again believed to be proper and not to constitute the insertion of new matter.

[5] Concerning the "same" or "different" materials recitation and the sandwich type formats in various claims, the following remarks are offered. Former

now canceled claims 34 and 35 recite that the solid support and system are composed of the same materials (claim 34) or different materials (claim 35). New claims 59-60 and 113-114 recite either that the solid support and the system are "composed of the same materials" (claims 59 and 113) or "composed of different materials" (claims 60 and 114). It should be pointed out that in the parent application (page 13, last paragraph, through page 14, first paragraph), Applicants disclose:

> Yet another aspect of the method of the present invention involves generating the soluble signal from the probe-analyte hybrid in a device capable of transmitting light therethrough for the detection of the signal by spectrophotometric techniques. Examples of devices useful in the spectrophotometric analysis of the signal include conventional apparatus employed in diagnostic laboratories, i.e., plastic or glass wells, tubes, cuvettes or arrangements of wells, tubes or cuvettes. It may also be desirable for both the solid support to which the analyte is fixed and the device to be composed *of the same material*, or for the device to function as the support in addition to facilitating spectro-photometric detection. [underline and italics added]

Moreover, claim 19 originally filed in the parent application (Serial No. 06/732,374) recites a method "characterized in that said solid support and said device are composed of the same materials." Thus, it is believed that the above-quoted disclosures and originally filed claims reasonable convey to the artisan that Applicants were in possession of the subject matter recited in claims 34 and 35 (new claims 59-60 and 113-114), namely, that the elements at hand could be composed of the same or of different materials in accordance with the present invention.

Concerning the recitations in former now canceled claims 36 and 37, Applicants note the following. Claim 36 called for "[t]he composition according to claim 27 wherein one of said oligonucleotide or polynucleotide strands is indirectly fixed or mobilized to the solid support". Claim 37 depended from claim 36 and recited "wherein said oligonucleotide or polynucleotide strand is indirectly fixed or immobilized to a solid support by sandwich hybridization." New claims 61-62 and 84-85 correspond in large part to former claims 36-37, respectively. The subject matter and language of the foregoing claims is well supported in the original

disclosure. For example, in the instant specification, page 10, first full paragraph, it is disclosed:

> In accordance with the practice of this invention, analytes in a biological sample are preferably denatured into a single-stranded form, and then directly fixed to a suitable solid support. Alternatively, the analyte may be directly fixed to the support in double-stranded form, and then denatured. The present invention also encompasses **indirect fixation** of the analyte, such as in in situ techniques where the cell is fixed to the support and **sandwich hybridization** techniques where the analyte is hybridized to a polynucleotide sequence that is fixed to the solid support. In the practices of this invention, it is preferred that the solid support to which the analyte is fixed be non-porous and transparent, such as glass, or alternatively, plastic, polystyrene, polyethylene, dextran, polypropylene and the like. Conventional porous materials, e.g., nitrocellulose filters, although less desirable for practice of the method of the present invention, may also be employed as a support. [bold added]

Thus, the subject matter of new claims 61-62 and 84-85 (as with former claims 36-37) is fully supported by the disclosure and is not new matter.

**[6]** Concerning the direct or indirect attachment of the signalling moiety to the chemical label in former claim 42 which depended from former claim 41, the following remarks are offered. Both claims have been canceled in favor of new claims 69-70 and 92-93, as well as 124-125.

Claims 69, 92 and 124 recite " . . . wherein said chemical label is indirectly attached to the oligonucleotide or polynucleotide through a bridging moiety." Claims 70, 93 and 125 recite " . . . wherein the soluble signal generating signalling moiety of said chemical label or labels is directly or indirectly attached thereto." In other words, the latter claims set forth the direct or indirect attachment of the signalling moiety (of the chemical label or labels); whereas the former claims are directed to the indirect attachment of the chemical label or labels to the sequence through a bridging moiety. These represent separate although by no means mutually exclusive embodiments for the present invention.

The subject matter of both sets of claims (69, 92, 124, and 70, 93 and 125) is well supported in the specification. A thorough discussion of the soluble signal

generating signalling moiety and its direct attachment or indirect attachment (for example, through a bridging moiety) is provided in the background section, beginning on page 5, first full paragraph and continuing through page 8, first two lines. More particularly, it is disclosed in these pages that:

- "A wide variety of signalling events may be employed to detect the occurrence of the primary recognition event" (page 5, lines 3-5);

- "[a]lthough the label may only consist of a signalling moiety, which may be detectable, it is more usual for the label to comprise a combination of a bridging moiety covalently or non-covalently bound to the polynucleotide sequence" (page 5, lines 9-13);

- "The combination of bridging moiety and signalling moiety . . . may be constructed before attachment or binding to the sequence, or it may be sequencially (sic) attached or bound to the sequence. . . [T]he bridging moiety may be first bound or attached to the sequence and then the signalling moiety combined with that bridging moiety. . . [or] or several bridging moieties and/or signalling moieties may be employed together in any one combination of bridging moiety and signalling moiety" (page 5, second full paragraph);

- "Covalent attachment of a signalling moiety or bridging moiety/signalling moiety combination to a sequence is exemplified by the chemical modification of the sequence with labels . . . that themselves provide signals . . . or the chemical modification of the sequence with at least one combination of bridging moiety and signalling moiety to provide that signal" (page 5, third full paragraph);

- "Non-covalent binding of a signalling moiety or bridging moiety/signalling moiety to a sequence involve the non-covalent binding to the sequence of a signalling moiety that itself can be detected by appropriate means . . . or the non-covalent binding to the sequence of a bridging moiety/signalling moiety to provide a signal that may be detected by one of those means . . . bound to an antibody, a fluorescent moiety or another moiety which is detectable by appropriate means. . ." (page 6, first paragraph); and

- ". . . All that is required is that the signalling moiety provide a signal that may be detected by appropriate means and that the bridging moiety, if any, be characterized by the ability to attach covalently or to bind non-covalently to the sequence and also the ability to combine with a signalling moiety" (page 6, first full paragraph).

The above-excerpted detailed discussion is followed by the disclosure beginning on page 12, last paragraph, continuing through page 13, first paragraph. There, it is disclosed:

> As another aspect of the present invention, the signalling moiety may be attached to the probe through the formation of bridging entity or complex. Likely candidates for such a bridging entity would include a biotin-avidin bridge, a biotin-streptavidin bridge, or a sugar-lectin bridge.
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> Once the fixed probe-analyte hybrid is formed, the method may further involve washing to separate any non-hybridized probes from the area of the support. The signalling moiety may also be attached to the probe through the bridging moiety after the washing step to preserve the materials employed. Thereafter, another washing step may be employed to separate free signalling moieties from those attached to the probe through the bridging moiety.

Thus, the subject matter of both sets of claims (69, 92, 124, and 70, 93 and 125) is well supported by the original disclosure.

**[7]** Concerning the apparatus of former now canceled claim 46 (new claim 100), as discussed in the opening remarks of this Amendment, the first element (1) in this claim has been amended to recite "one or more solution containing means, each comprising a transparent non-porous or translucent non-porous device . . ." It is respectfully submitted that the original disclosure supports both the feature where a single device is claimed as well as a plurality of devices. For example, in the specification, page 13, last paragraph, through page 14, first line, it is disclosed that "[y]et another aspect of the method of the present invention involves generating the soluble signal from the probe-analyte hybrid in a device capable of transmitting light therethrough for the detection of the signal by spectrophotometric techniques. Examples of devices useful in the spectrophotometric analysis of the signal include conventional apparatus employed in diagnostic laboratories, i.e., plastic or glass wells, tubes, cuvettes or arrangements of wells, tubes or cuvettes." See also page 14, second paragraph, particularly lines 3-5 and 10-21 in that second paragraph. Therefore, the subject matter of new claim 100 is fully supported by the original disclosure.

**[8]** Regarding the open claim language term "comprising" in former claim 48 (line 2), as noted above, this claim has been replaced by new claim 102 which in contrast to its predecessor, calls for a three component - not two component - system that is transparent non-porous or translucent non-porous and is capable of retaining or containing a fluid or solution. The first component is (i) an oligonucleotide or polynucleotide capable of hybridizing to the oligo- or

polynucleotide sequence and comprising a label or labels which comprise a signalling moiety or moieties which are capable of generating a soluble signal. The second component is (ii) a solid support capable of directly or indirectly fixing or immobilizing the oligo- or polynucleotide sequence or the oligonucleotide or polynucleotide (i) [the first component]. The third component in the system recited in claim 102 is (iii) fluid or solution. Applicants acknowledge the statements in the January 5, 1995 Office Action to the effect that the embodiments on page 14 in the specification call for a polynucleotide being fixed on the solid support as cited in claim 24 as filed. Again, Applicants respectfully point out that page 14 and original claims 24-26 are but isolated portions of the disclosure, and that to do justice to the present claims including new claim 102+, resort to the rest of the disclosure must be made. For example, on page 10 in the instant specification, it is disclosed:

> In accordance with the practice of this invention, analytes in a biological sample are preferably denatured into single-stranded form, and then directly fixed to a suitable solid support. Alternatively, the analyte may be directly fixed to the support in double-stranded form, and then denatured. The present invention also encompasses indirect fixation of the analyte, such as in in situ techniques where the cell is fixed to the support and sandwich hybridization techniques where the analyte is hybridized to the polynucleotide sequence that is fixed to the solid support. In the practices of this invention, it is preferred that the solid support to which the analyte is fixed be non-porous and transparent. . .
>
> It is also highly desirable that the analyte be easily fixed to the solid support. The *capability to easily fix the analyte to a transparent substrate* would permit rapid testing of numerous samples by the detection techniques described herein. [underline and italic added]

In the DETAILED DESCRIPTION section (page 15, second paragraph), the examples are said to be illustrative of preferred embodiments of the method of the present invention. "Specifically referred to therein are methods for fixing the analyte to a non-porous solid support . . ." It is abundantly clear that the disclosure supports the claimed subject matter wherein a support is capable of fixing or immobilizing an oligonucleotide or polynucleotide sequence as set forth in new claim 102.

With respect to the solid support being capable of fixing or immobilizing the oligonucleotide or polynucleotide (i) in claim 102, this embodiment is likewise well supported by the disclosure. In EXAMPLE 5 (page 20, first full paragraph), Applicants disclose:

> The advantages of the practices of this invention are also obtainable when the probe is immobilized on a non-porous plastic surface. When a plastic surface is employed, it is sometimes desirable to increase the effectiveness or uniformity of the fixation by pretreating the plastic surface.

Later in EXAMPLE 6 (page 21, last paragraph, through page 22, first paragraph), Applicants describe a labelled, non-biotinylated denatured DNA that was applied to DDA-coated polystyrene plates.

It is quite clear, therefore, that the capability of the solid support in claim 102 to fix or immobilize either the oligonucleotide or polynucleotide sequence or the oligonucleotide or polynucleotide (i) is fully supported by the above-quoted portions in the specification.

**[9]** An issue was raised earlier regarding a separate solid support (with no limitations on porosity or transparency) as a subelement in a transparent non-porous or translucent non-porous system. This issue was specifically directed to former claims 48 and 49 whose entry was denied. New claims 48-49 and 102-103 added above correspond by and large to the aforementioned non-entered claims 48 and 49. In response to this issue, Applicants wish to point out that the disclosure fully supports this embodiment such that the artisan would readily recognize it to be in their possession. The term "system" is supported in the disclosure. See specification, page 28, first paragraph, and originally filed claim 71, discussed *supra*, at page 24, last paragraph, through page 25, first two lines. In addition, the disclosure is clear that the solid support may take any number of possible forms, including a form not limited by porosity or transparency.

Thus, the disclosure fully supports the subject matter set forth in new claims 48-49 and 102-103. For example, on page 10, first full paragraph, *supra* (see also this Amendment, page 26, last paragraph, through page 27, first full paragraph) the disclosure refers to direct and indirect fixation of analytes to a "suitable solid support." Moreover, the disclosure is clear in its statements that a non-porous translucent or transparent support was preferred. See page 10, lines 18-20; page 14, third and fourth lines from the bottom of the page; and page 15, first three lines under EXAMPLE 1.

**[10]** As in the case of **[5]** above, former non-entered claims 53 and 54 were rejected for new matter on the basis of the "same" or "different" limitations. These former non-entered claims have been replaced with new claims 60 and 61, respectively. The basis in the disclosure for the "same" and "different" limitations in new claims 59-60 and 113-114, respectively, is set forth above in the discussion of **[5]** (see this Amendment, page 19, last paragraph, through page 21, first two lines).

**[11]** An issue of new matter was raised with respect to former apparatus claim 68 that has been replaced by new claim 132, the latter calling for "one or more transparent non-porous or translucent non-porous" devices. Applicants' earlier remarks above in **[7]** also dealt with the "plurality of devices" and indicated the basis in the disclosure for both a single device and a plurality of devices.

In light of the foregoing remarks, the rejection for new matter is believed to have been obviated. Entry of all the claims, 48-132, is respectfully urged.

**The Rejection Under 35 U.S.C. §112, Second Paragraph**

Claims 31-33, stand rejected for indefiniteness under 35 U.S.C. §112 second paragraph. In the Office Action, (pages 4-5) the Examiner stated:

> Claims 31-33 are rejected under 35 U.S.C. § second paragraph as being indefinite for failing to particularly point and distinctly claim the subject matter which applicant regards as the invention.
>
> Claim 31 is unclear in that what appears to be a Markush group lists plastic as well as polystyrene, dextran, and polypropylene because polystyrene, dextran, and polypropylene are plastics. What are the metes and bounds of the claim unless it is merely is inclusive of any plastic?
>
> Claims 32 and 33 are vague and indefinite in that the cooperativity between the members listed therein and the solid support of the "system" is not defined. Seemingly the cited members of claims 32 and 33 are meant to be options for the solid support of claim 27 but this is not clearly defined as such. Could applicants mean the solid support and the members to be separate components of the claimed "system"? Clarification is requested as to the metes and bounds and relationships of the components of the claimed "system".

The indefiniteness rejection is respectfully traversed.

As indicated in the opening remarks of this Amendment (see page 17, last paragraph, through page 18, first paragraph), the clarity with respect to the Markush members for the solid support and system has been addressed in various new claims. As represented in the new claims, Markush recitations are provided for the porous solid support (claims 51-52 and 105-106); the non-porous solid support (claims 53-55 and 107-109); and the non-porous system (claims 78-80). In particular, the porous polymeric materials (dextran, cellulose and nitrocellulose are now segregated from the non-porous polymeric materials (polyethylene, polypropylene, polystyrene and epoxy). As discussed in the opening remarks, support for the terms "cellulose" and "nitrocellulose" in the new dependent claims is found in original claims 45 and 46, respectively for "polyethylene," support in the new dependent claims is found in original claim 43. For "epoxy," support is taken from the first originally-filed specification (page 31, last paragraph) and original claim 66. The former Markush members in claim 31 have been placed in new claims 54, 79 and 108 ("siliceous material comprises glass or a glass-coated surface"); new claims 52 and 106 ("said porous polymeric materials is selected from . . . dextran, cellulose and nitrocellulose"); and new claims 55, 80 and 109 ("said non-porous polymeric material is selected from . . . polyethylene, polypropylene, polystyrene and epoxy").

Regarding the cited members in former now canceled claims 32 and 33, Applicants have addressed this issue in various new claims above. In former claim 32, the system was selected from the Markush members listed therein; whereas former claim 33 recited that the "well comprises a microtiter well." New claims 56, 81 and 110 correspond to former claim 32. New claims 57-58, 82-83 and 111-112 correspond in part to former claim 33 with the following exceptions. In new claims 57, 82 and 111, "said well comprises a microtiter well;" whereas new claims 58, 83 and 112 recite that "said wells in the apparatus comprise microtiter wells." These changes in the foregoing new claims are discussed in the opening remarks of this Amendment (page 18, last paragraph). It is believed that the foredescribed amendments obviate the indefiniteness rejection to claims 32 and 33.

Reconsideration and withdrawal of the indefiniteness rejection is respectfully requested.

**The Art-Based Rejections**

Before addressing the issues under 35 U.S.C. §102, Applicants would like to address by way of introduction the very significant and patentable distinctions between the prior art methodology of *in situ* hybridization on the one hand, and the instant invention and its reliance on the generation of a soluble signal on the other. The former is the subject matter of the three documents cited in the rejections discussed below, *supra*.

*In situ* hybridization is clearly a very specialized case of nucleic acid hybridization. As pointed out by Piper and Unger in their book, Nucleic Acid Probes: A Primer for Pathologists [ASCP Press, American Society of Clinical Pathologists, Chicago, 1989, Chapter 2, "Nucleic Acid Hybridization Analyses and Other Nucleic Acid Assays," pages 61-66), *in situ* hybridization:

> . . . *involves taking morphologically intact tissues, cells, or chromosomes through the hybridization process to demonstrate not only the presence of a particular piece of genetic information, but also its* ***specific location*** *within the tissue, cell, or chromosomes*. In situ hybridization techniques represent the best compromise between making the nucleic acid target available for hybridization *and maintaining the* ***morphologic integrity*** *of the starting material*. The target nucleic acids are found intimately mixed with the proteins, other nucleic acids, and membranes that form the basis of the familiar staining patters with "routine" stains such as H&E, Papanicolaou, or Giemsa banding. The goal is to make the target nucleic acid available for *hybridization while* ***maintaining a recognizable "staining environment"*** *so that the tissue, cell, or chromosome can be identified by* ***the landmarks of routine staining***. [pages 61-62, emphasis added]

With *in situ* hybridization, the methods for affixation of the sample starting material to the viewing slide is critical. Piper and Unger explain:

> Sample preparation involves some method of affixing the starting material to a microscope slide, *because* ***light microscopic examination is required*** *to evaluate the assay*. The slide becomes the solid support that carries the cells, tissue, or chromosomes through all the following steps of the hybridization assay. ***This step is not trivial*** *because the conditions of the hybridization assay can be quite harsh and loss of*

> *sample through detachment during the assay is a major concern.* Adherence is improved by coating the glass with substances such as gelatin, polylysine, aminopropylsilane, or Elmer's Glue .
>
> . . .
>
> The sample type and method of preparation greatly influence the details of the technique. Fresh or frozen material will behave much differently from fixed material, particularly if a cross-linking fixative such as formaldehyde or glutaraldehyde is used. *Fixatives were developed to* ***preserve morphology*** *through routine staining procedures, and thus will add to the* ***morphologic integrity*** *of the finished hybridization product*. At the same time, fixatives will decrease the availability of the nucleic acid that is to undergo hybridization. [pages 62-63, emphasis added]

Because morphology, morphologic integrity and landmarks are vital to *in situ* hybridization, *supra*, labelling and sample treatment are crucial to evaluating and interpreting *in situ* results. In the case of labels, Piper and Unger elaborate:

> Detection of the hybridization signal depends on the method used to label the probe. Various radioactive labels may be used; all are detected with silver emulsion autoradiography. Exposure times range from overnight to several months. With low-energy radioisotopes such as tritium, there is ***minimal scattering*** *of the silver grains from the site of probe localization* but the required exposure times are the longest. High-energy isotopes such as $^{125}I$ give very poor localization of signal but require very short development times. In practice, $^{35}S$ is a useful compromise between signal localization and development time.
>
> With nonradioactive labels the final detection is analogous to methods used in immunohistochemistry: either the fluorescent tags or histochemical enzymes may be utilized *to* ***localize*** *the position of the reporter molecules on the probe*. *There is essentially* ***no scatter of signal*** *with either method*. The results of fluorescence may be viewed immediately, while enzyme-derived colorimetric products require 10 minutes to overnight for development. Colorimetric or fluorescent analyses are generally much faster than autoradiography, but many investigators believe there is a signficant loss of sensitivity. Others feel that with careful optimization of the assay conditions, nonradioactive methods can achieve sensitivities very close to those obtained with radioactive isotopes. [page 65, emphasis added]

Regarding evaluation and interpretation of *in situ* results, Piper and Unger explain:

> After counterstaining *to bring out the* ***morphology*** *and maximize contrast between the signal and surroundings, the final product is evaluated by light or fluorescence microscopy*. *Through visual inspection the presence of signal is determined qualitatively, and it* ***is localized to a particular cell or chromosome***. Autoradiographic detection can be made semiquantitative by counting the number of developed silver grains per area. Semiquantitative colorimetry can be accomplished with computer-assisted image analysis and morphometry.

> Attempts at quantitation can be quite complicated, and most investigators use in situ hybridization at the qualitative level.
>
> *In situ hybridization occurs and is **interpreted in a morphologic context***. Unlike hybridization assays based on extraction techniques, the sensitivity of the in situ assay is influenced not only by the number of copies of the target sequence but also by its distribution in a sample, *while in situ methods are most sensitive when target sequences are nonuniformly distributed*. When only one or two cells in a relatively large sample contain the target sequences, extraction methods dilute those positive sequences, while in situ hybridization assays preserve the natural concentration and easily detect the few positive cells in a negative background. This is the only form of hybridization that combines the power of morphologic analysis with a sophisticated genetic analysis and *permits **definitive localization** of genetic information*. [page 66, emphasis added]

*In situ* hybridization is generally unsuitable for handling large numbers of samples. In closing the section, Piper and Unger note:

> The *in situ hybridization assay can be quite **tedious** and each sample must be **treated individually***. *This makes the handling of multiple samples very difficult and is one of the biggest drawbacks of the assay. Because **morphologic integrity** is crucial to the assay, investigators familiar with extraction-based techniques often find in situ hybridization to be a kind of "black magic" that is subject to many more variables influencing both signal and background*. With experience in working with tissues, cells, and chromosomes, these difficulties usually can be resolved; however, the assay does have an extra level of complication compared with extraction-based techniques. [page 66, emphasis added]

For the Examiner's convenience, a copy of pages 61-66 from Piper and Unger's above-quoted book is attached as EXHIBIT 8.

In summary, *in situ* hybridization can only be practiced in the context of clear boundaries and well-defined morphology against which a localized signal must be produced and interpreted.

In contrast to *in situ* hybridization, the instant invention provides for compositions of matter (claims 48-99), an apparatus (claims 100 and 132), a kit (claim 101) and a transparent non-porous or translucent non-porous system (claims 102-131), all of which are based upon the generation of a soluble signal. With soluble signals as set forth in the instant invention, the signal is not localized and morphology is neither required, maintained or viewed. Indeed, with the generation of

a soluble signal, a dispersed or scattered signal in solution is obtained *without* any regard to morphologic integrity.

Thus, with its preoccupation on morphologic integrity (landmarks, boundaries and the like) and a localized signal with minimal scattering, the prior art *in situ* hybridization actually teaches away from the instant invention and the generation of soluble signals which require neither a localized signal nor morphologic integrity. In fact, *in situ* hybridization is so different from soluble signal generation that *in situ* hybridization actually represents non-analogous art to the instant field of soluble signal nucleic acid assays which was pioneered by the instant inventors.

Before addressing the three rejection under §102, Applicants also wish to point out that the instant assignee and its parent company have successfully commercialized its soluble signal technology which is also called the microplate assay. The Enzo Microplate Assay (its tradename) was developed in the late 1980s and has since then been marketed and sold or distributed worldwide. This product has been successfully applied to a number of pathogens, including HIV-1, HIV-2, Microbacterium tuberculosis (MTB), and Hepatitis B. Attached to this Amendment are copies of product inserts relating to Enzo's HIV-1 Microplate Hybridization Assay (EXHIBIT 9), HIV-2 Microplate Hybridization Assay (EXHIBIT 10), MTB Microplate Hybridization Assay (EXHIBIT 11) and Hepatitis B Microplate Hybridization Assay (three separate product inserts in EXHIBIT 12). With respect to detecting HIV-1, a presentation was given and an abstract was published at the 1990 Annual Meeting of the United States and Canadian Academy of Pathology (copy attached as EXHIBIT 13). More recent product brochures for The Enzo Microplate Assay for HIV DNA are attached as EXHIBITS 14 and 15, respectively. For M. tuberculosis detection, a slide session was given and an abstract was published in conjunction with the 1988 Annual Meeting of the American Society for Microbiology (ASM) (copy of materials attached as EXHIBIT 16). More recently in 1994, a presentation was given and a poster abstract was published at The San Diego Conference - The Genetic Revolution (EXHIBIT 17).

Copies of pages from the instant assignee's 1992 and 1995 product catalogs relating to the Enzo Microplate Assay are attached as EXHIBITS 18 and 19, respectively.

Turning now to the three anticipation rejections . . .

## I. The First Rejection Under 35 U.S.C. §102

Claims 27-32, 34-36, 38, 39 and 41-45 stand rejected under 35 U.S.C. §102(e) as being clearly anticipated by Stuart et al., U.S. Patent No. 4,732,847. In the Office Action (pages 5-6), the Examiner stated:

> Stuart et al. disclose the formation of DNA-RNA hybrids by in situ hybridization performed on microscope slides wherein the hybrids are detected by antibody binding to said hybrids starting in column 4, line 1, and proceeding through the section entitled "EXPERIMENTAL". Several types of antibody label types are given in Stuart et al. in column 4, lines 37-55, inclusive of enzymes such as horseradish peroxidase. These disclosures read on the above listed instant claims. It is additionally noted that microscope slides are non-porous and transparent and are disclosed as being viewed by light microscopy as summarized in column 6, lines 17-26, thus also inclusive of a "system" as instantly claimed. Such a light microscope system includes nonporous transparent elements which are interpretable as reading on these limitations of the system of the instant claims. it is also noted that Stuart et al. reads on the above listed instant claims after removal of the NEW MATTER discussed above. The above listed claims contain the limitation directed to "a signalling moiety which is capable of generating a soluble signal". Enzymes such as horseradish peroxidase inherently are "capable of generating a soluble signal" as is well known in the peroxidase labeled antibody art. It is acknowledged that Stuart et al. does not disclose the actual generation of a soluble signal. This lack of actual soluble signal generation is, however, not deemed to prevent this rejection because the claims are composition claims citing only the "capability" of generating such a soluble signal. The inherency of this "capability" has been discussed above.

The anticipation rejection over Stuart et al. is respectfully traversed.

As a document cited for anticipation, Stuart's patent lacks two material elements in the instantly claimed invention.

**First**, Stuart et al. is wholly and only concerned with *in situ* hybridization which requires morphologic integrity and a localized signal. More particularly, Stuart et al. disclose *in situ* hybridization to form a hybrid DNA-RNA duplex between a nucleic acid sequence (DNA or RNA) fixed to a solid support and another type of nucleic acid (RNA or DNA) having a predetermined sequence to bind to a homologous fixed sequence. Having obtained such a hybrid duplex through *in situ* hybridization, Stuart then uses labeled hybrid duplex monoclonal antibodies (or labeled antibodies which bind to the "anticomplex") to detect the presence of the fixed specific nucleic acid sequenc. See Stuart et al., column 1, lines 65-68; column 3, lines 41-42; column 4, lines 24-35; column 5, lines 2-4 (in the EXPERIMENTAL section); column 5, lines 60-68 (in the subsection "In Situ Hybridization"); and column 7, lines 14-16.

Because it is only concerned with *in situ* hybridization, Stuart's patent not only teaches away from the instant invention and soluble signalling, but it also represents non-analogous art that is inapplicable to the invention at hand. See the preceding discussion on *in situ* hybridization (this Amendment, page 34, second paragraph, through page 37, first full paragraph).

**Second**, unlike the instant invention which calls for and requires chemically labeled nucleic acid, Stuart et al. do not disclose or even suggest that any nucleic acid could or should be labeled. Instead, Stuart et al. only label the monoclonal antibodies (or antibodies in the case of the "anticomplex") in their disclosed methods and kits. See, for example, column 4, lines 24-55 in Stuart's patent where it is disclosed:

> The identification of the presence of the hybrids may now be achieved by employing monoclonal antibodies specific for the hybrid complex. Detection can be achieved *by labeling either the monoclonal antibody specific for the hybrid DNA-RNA complex,* hereinafter referred to as "anticomplex" or *by employing labeled antibodies which bind to the anticomplex*. For example, where the monoclonal antibody is derived from a mouse, antibodies to mouse antibodies e.g. rabbit anti(-mouse IgG), could be labeled so as to bind to any anticomplex bound to the complex bound to the solid support.
>
> A wide variety of labels have been used in other environments which would be applicable here. One of the more common labels is radionuclides, which can be used with autoradiography to visualize the areas of binding. Another label is a fluorescer e.g. fluorescein,

> merocyanine, rhodamine, etc., which by irradiation with light of excitation, the presence of fluorescence can be monitored. Alternatively, an enzyme can be used which results in a product which can be detected and localized in the area of the enzyme. A large number of dyes or metals capable of reduction can be employed to provide detection. Common enzymes include horseradish peroxidase, glucose oxidase, β-galactosidase, or the like. The particular label or manner in which the detectable signal is observed is not critical to this invention. Evidently, by employing antibodies to this anticomplex, the number of labels associated with a particular binding of the anticomplex to the complex can be greatly amplified.
>
> [underline and italic added]

Later in their example, Stuart et al. disclose the identification of hybrids by secondary immunofluorescence. More specifically, they disclose that "[g]oat anti-rabbit and goat anti-mouse Ig antisera conjugated with fluorescein isothiocyanate were purchased from Antibodies, Inc." [column 6, lines 19-21]. Furthermore, "[w]hen the RNA normally present in the polytene preparation was not predigested with Rnase A, multiple fluorescent bands were observed after hybridization. This evidences that the antibodies were not reactive with chromosomal DNA but were able to bind to RNA-DNA duplexes" [column 6, lines 31-36]. See also claim 5 in Stuart et al. (. . . determining the presence of monoclonal antibody bound to said solid support by means of a label providing a detectable signal, which label is bound directly or indirectly to said monoclonal antibody), and claim 14 (kit for determining the presence in situ of a hybrid DNA-RNA complex . . . said secondary antibodies having a label capable of providing a detectable signal).

Thus, it is quite plain that Stuart et al. only contemplate labeling the monoclonal antibodies (or antibodies in the case of the "anticomplex"). Such labelling is in contrast to the instant invention in which the oligonucleotides or polynucleotides are chemically labeled.

In view of Stuart's lack of identity with respect to at least two instantly claimed material elements, Applicants respectfully request reconsideration and withdrawal of the anticipation rejection.

## 2. The Second Rejection Under 35 U.S.C. §102

Claims 27-31, 34-36, 38, 39, and 41-45 are rejected under 35 U.S.C. § 102(a) as being clearly anticipated either by Langer-Safer et al. ["Immunological method for mapping genes on *Drosophila* polytene chromosomes," Proc. Natl. Acad. Sci.(USA) 79:4381-4385 (1982) or Manuelidis et al. ["High-resolution Mapping of Satellite DNA Using Biotin-labeled DNA Probes," Journal of Cell Biology 95:619-625 (1982)]. On page 7 of the Office Action, the Examiner stated:

> The listed claims are anticipated either by Langer-Safer et al. or Manuelidis et al. in the same manner as the above rejection based on Stuart et al. because both references also discussed the performance of in situ hybridization of chromosome spreads on microscope slides etc. as summarized above. It is noted that Langer-Safer et al. has been listed on a PTO Form 1449, filed by applicants on 6/2/94, as Langer et al.(1982) as given in PNAS, volume 79, pages 4381-4385. The correct citation is to Langer-Safer et al. and has also been corrected on the PTO Form 1449.

At the outset, Applicants gratefully acknowledge the correction of the Langer-Safer et al. citation in Applicants' Information Disclosure Statement filed on June 2, 1994.

As in the case of the previous anticipation rejection based on Stuart et al., Applicants note that both Langer-Safer et al. and Manuelidis et al. are confined to *in situ* hybridization. As such, both teach away from the instant invention and furthermore, both represent non-analogous art to the invention at hand and soluble signalling. As discussed above (see this Amendment, page 34, second paragraph, through page 37, first full paragraph), *in situ* hybridization requires morphologic integrity and localized signal production - and both requirements are inapposite to soluble signal generation as embodied in the instant invention.

This distinction between *in situ* hybridization, immunoprecipitates and insoluble signal on the one hand, and the instantly recited soluble signal generation on the other, cannot be overemphasized, nor should it in any way be overlooked or minimized. When performing *in situ* hybridization, the technician or researcher is

looking under the microscope and observing form or morphology as well as signalling events within the context of any such form or morphology. Such a person only observes and amasses information within the context of clearly defined boundaries and visible shapes. The cells or the contents of cells under examination must have such boundaries and shapes in order to carry out detection in *in situ* hybridization. Separation and analytical techniques are largely based on precipitates and defined boundaries. With soluble signals, however, the approach is entirely antithetical to the purposes of and the information being sought through light microscopic examination. With soluble signals, however, there are no clearly defined boundaries, shapes and morphology with which the technician or researcher must contend. Thus, *in situ* hybridization as disclosed in the Langer-Safer and Manuelidis et al. documents teaches away from the instant invention. By directing and focusing attention to morphology and localized signaling, both of which are required in *in situ* hybridization, Langer-Safter et al. and Manuelidis et al. actually discourage any resort to soluble signals. Indeed, for the skilled artisan to go from precipitates and insoluble signals to the instantly claimed soluble signal generation, all prior conceptions and experiences relating to detection (localized signals) and observation (morphologic integrity) must be entirely revised if not altogether jettisoned.

Reconsideration and withdrawal of the rejection based on anticipation by Langer-Safer et al. or Manuelidis et al. is respectfully requested.

3. **The Third Rejection Under 35 U.S.C. §102**

Claims 27-31, 34-36, and 38-45 are rejected under 35 U.S.C. § 102(e) as being anticipated by Ward et al., U.S. Patent No. 4,711,955. In the Office Action (page 7), the Examiner stated:

> Ward et al. disclose via the "GENERAL PROTOCOL" and with connected discussion elsewhere at the bottom of columns 19 and 20 in situ hybridization where immobilized double-stranded nucleic acid is shown visualized with a biotinylated probe bound to avidin-peroxidase. This reads on the listed claims as does the above disclosures directed to in situ hybridization but also covering biotin-avidin mediated embodiments.

The rejection for anticipation by Ward et al. is respectfully traversed.

As in the case of the other documents cited above for anticipation, Ward et al. is also wholly concerned with immunoprecipates, precipitated signals, insoluble colored precipitates and the like. Regarding Ward's "GENERAL PROTOCOL," it is quite clear that the patentees are working with the enzyme peroxidase in what are clearly insoluble precipitates for light microscope visualization. In column 24, lines 33-62, Ward et al. disclose:

> An alternative to the fluorescence method for visualizing hybridized probes is to direct enzymes such as peroxidase, alkaline phosphatase of[or] β-galactosidase to the hybridization site where enzymatic conversion of soluble substrates to *insoluble colored precipitates* permits light microscope examination. The important advantage of this technique is that the histochemical methods are 10 to 100-fold more sensitive than fluorescence detection. In addition, the *colored precipitates* do not bleach with extensive light exposure thus avoiding one of the general disadvantages of fluorescent light microscopy. These enzymes can be coupled to the final antibody instead of using bifunctional reagents such as glutaraldehyde or in the case of peroxidase via oxidation of the peroxidase carbohydrate moieties to aldehydes and coupling of these residues with ε-amino groups of the desired protein. For the streptavidin-biotinized carrier protein method, an enzyme with biotinyl groups coupled to it could replace a fluorescently-biotinized carrier system. Alternatively, the enzyme could be coupled via biotin to the last layer of streptavidin with amplification of streptavidin sites being built up in preceding layers using biotinized BSA or thyroglobulin. We will begin developing the necessary histochemical reagents and the appropriate *combinations substrate/insoluble product combinations* for visualizing in situ hybridizations without background problems in the near future. The histochemical approaches to signal amplification should therefore be ready for trial in the summer of 1981. [underline and italic added]

Thus, the focus of Ward et al. is on insoluble colored precipitates and direct light microscopic visualization which are diametrically opposed to and actually teach away from the instant invention and the notion of soluble signal generation. See discussion above relating to *in situ* hybridization (page 34, second paragraph, through page 37, first full paragraph), as well as the the other two rejections under §102.

Reconsideration and withdrawal of the third anticipation rejection based upon Ward et al. is respectfully requested.

Applicants sincerely appreciate the indication from the Examiner (Office Action, pages 7-8) that claims 32, 33, 37, 46, and 47 are allowable over the prior art of record "because the prior art of record does not teach or suggest the well, tube, or cuvette practice as solid supports for enzyme labeled hybridization nor sandwich hybridization used for immobilizing a probe/target hybrid." It is believed, however, that the above-submitted new claims, foregoing remarks and attached exhibits (1-19), place all of the instant claims, 48-132, in allowable condition.

* * * * * * *

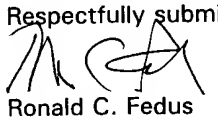
## SUMMARY AND CONCLUSIONS

New claims 48-132 are presented for further examination. Their predecessor claims, 27-47, have been canceled.

The fee for adding new claims 48-132 is $2,144.00, based upon the cost of $156.00 for 2 additional independent claims (in excess of the three previously paid for); $250.00 for the first presentation of multiple dependent claims; and $1,738.00 for 79 claims presented in excess of the 21 previously paid for claims [79 x 22 = $1,738.00]. The Patent and Trademark Office is hereby authorized to charge the amount of $2,144.00 to Deposit Account 05-1135. This Amendment is also accompanied by a Petition to Revive an Unintentionally Abandoned Application Under 37 C.F.R. §1.137(b) and authorization for the large entity fee therefor. If any other fee or fees are due in connection with this Amendment or the Petition request, authorization is further given to charge the amount of any such fee or fee(s) to Deposit Account No. 05-1135, or to credit overpayment thereto.

In view of the above discussion of the issues, submission of new claims, and attached exhibits (1-19), Applicants respectfully submit that all of the instantly presented claims (48-132) are in allowable condition. Should it be deemed helpful or necessary, the Examiner is respectfully invited to telephone the undersigned at (212) 856-0876 to discuss the subject application.

Respectfully submitted,



Ronald C. Fedus
Registration No. 32,567
Attorney for Applicants

**ENZO DIAGNOSTICS, INC.**
**c/o Enzo Biochem, Inc.**
**575 Fifth Avenue, 18th Floor**
**New York, New York 10017**
**Tel (212) 856-0876**